

REARING THE PAINTED LADY *CYNTHIA CARDUI* L.  
WITH PARTICULAR REFERENCE TO THE USE OF  
SEMISYNTHETIC DIET

By BRIAN O. C. GARDINER\*

(Concluded from p168)

**The semisynthetic diet and its preparation**

When faced with trying to establish a species on a semisynthetic diet the first choice is to use one which has already been shown to be successful, either with a similar related species, or which is known to be accepted by a range of disparate species, and this was done with the painted ladies, the first choice being the diets used for *P. brassicae* and for *Manduca sexta*. Both of these have been shown to be acceptable to a wide range of Lepidoptera. (See Gardiner 1978 for both range and diet formula). Although some of the larvae started to eat on both these, neither the initial percentage starting to feed, nor the survival rate was acceptable and therefore a new formulation was made using, since the colony was already flourishing so well on nettle, dried nettle leaf in the diet. This the larvae at once took to and, much to my surprise, would switch to eating the diet even after they had been fed on nettle for their first two or three instars. Now this is something that neither *P. brassicae* nor *M. sexta* would ever do. Once they had tasted the real thing they will not accept diet. (David & Gardiner 1966 & personal observation).

The formulation finally settled on is basically that which has been in use over many years for rearing *P. brassicae*. The main change is the substitution of dried nettle leaf (which is a commercial product sold as "nettle powder") for the dried powdered cabbage leaves or sinigrin flavouring required by the cabbage white. For simplicity of making up the diet, the quantities of the ingredients were varied slightly from previous published formulae (see Gardiner 1978) to give rounded whole quantities for measuring out. The basic formula is presented in Table 2 and that of the vitamins used in Table 3. All the ingredients should be obtainable either through your local chemist or, if not, they are available from Messrs. Philip Harris Biological, Oldmixon, Weston-super-Mare, Avon BS24 9BJ. It may well prove that the proprietary vitamin supplement for small animals sold under the trade name "Vionate" and added at the rate of 0.5 or even 1 gram per batch is equally suitable as a substitute for the vitamins given in Table 3. I have used it with success on other species, but not carried out a definitive investigation as to its equal efficacy with the painted ladies.

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Water.....	600 ml
Agar.....	27 g
Water.....	330 ml
Casein (light,white).....	40 g
Wheat germ (Bemax).....	40 g
Sugar (white,domestic).....	40 g
Cellulose powder (Whatman CF11)....	6 g
Nettle powder.....	12 g
Wessons salts (salt mixture 'W')...	10 g
Inositol.....	1 g
Cholesterol.....	1 g
Potassium hydroxide 20%.....	5 ml
Choline chloride 10%.....	10 ml
Methyl-4-hydroxy benzoate (15% in alcohol).....	10 ml
Linseed oil.....	3 ml
Formaldehyde 10%.....	5 ml
Vitamin solution.....	2 ml
Vitamin C (ascorbic acid).....	5 g
Veterinary Aureomycin.....	3 g

Table 2: Ingredients to make 1.1 litres of semi synthetic diet for painted lady larvae.

Nicotinic acid .....	5.00 g
(+)-Pantothenic acid calcium salt.	5.00 g
Riboflavin (B2) .....	2.50 g
Aneurine hydrochloride (B1) .....	1.25 g
Pyridoxine hydrochloride (B6) ....	1.25 g
Folic acid .....	1.25 g
D-Biotin .....	0.10 g
Cyanocobalamin (B12) .....	0.01 g
<u>Mix well, weigh 2.0 g. of the mixture, and add to :</u>	
Water .....	50.00 ml
Alcohol (95% or absolute) .....	50.00 ml

Table 3: Composition of the vitamin mixture used in the semi synthetic diet. When made up, keeps indefinitely under refrigeration. Nomenclature according to, and ingredients available from, British Drug Houses (BDH Ltd.).

The diet is made up as follows:—

The agar is added to the first aliquot of cold water, brought to the boil and kept simmering for about ten minutes. Since it is very liable to burning, the boiling is best done using a double (porridge) saucepan. Failing that stand with it stirring slowly and continuously with a wooden spatula type kitchen spoon. It should now be put aside and allowed to cool to about 70-75° C. While cooling the potassium hydroxide is added to the other portion of water followed by most of the solid ingredients (except the vitamin C and antibiotic which are destroyed by over-heating) which are mixed in using a Kenwood chef, Moulinex or other similar kitchen blender. It matters not whether they be added one by one or all together, but there is something to be said for mixing in the casein first and then adding all the other solids together. For those whose wives may not possess such an implement, although it is much harder work, the diet can be mixed entirely by hand using an egg-beater or even a whisk. I have used both methods on several occasions in order to satisfy myself that the resulting diet is just as acceptable as the machine mixed version and while one has to be more careful in order to avoid lumps, it is. The reason for the potassium hydroxide is that it reacts with the casein (which is rather insoluble) to form potassium caseinate, a more soluble substance and it will be observed as the mixing progresses that it gradually thickens.

When all the solids have been mixed in, the liquid ingredients are now blended in and for measuring these a pipette, hypodermic syringe or small measuring cylinder may be used. Next the cooled down but still hot agar is added. Now for the quantities given the average kitchen blender may be too small, so add half the agar and then pour back the contents of the blender into the agar saucepan and mix in there using a wooden spoon or spatula. Finally pour half back into the blender and add the vitamin C and antibiotic. Again pour back into the saucepan and finish off the mixing with the wooden spoon. The diet is now poured into a suitably sized container and allowed to set.

For use the diet is going to be required as strips and as cubes of about 1½ cms. Since one is unlikely to require the entire batch made up immediately and since it does not do frozen diet any good at all to be frequently thawed out in order just to cut off and use a portion, once set the diet should be cut up into suitable slices which are separately wrapped in aluminium foil or polythene bags before being placed under refrigeration or deepfrozen.

The storage life of the diet in use at room temperatures is about a week or ten days. At refrigerator temperatures of 4°C it keeps for 3 weeks and in a deepfreeze at -25°C from 6-12 months. Although in theory storage should be almost indefinite, in practice it was found that the water gradually elutes out of the diet and forms ice

crystals on its surface. This happens even when it is tightly contained. Diet from the deepfreeze should be allowed to thaw out overnight at room temperature (or even in the refrigerator). It should never be thawed by running hot water over it or otherwise heating. When rearing larvae on diet, therefore, thought needs to be given to the following days requirements.

### **Rearing on the diet**

As was found when rearing on plants, so too with the diet, mortality was high when the larvae were kept in the least degree crowded, but minimal when not more than 10 or 12 early instars were kept together and then, from the third instar onwards, placed individually (or sometimes in pairs). Two methods of presentation were finally adopted, the first being used for the early instars and the second for the larger larvae. In the first a strip of diet about 1 cm wide was laid across the centre of a 9 cm diameter petri dish. The top of strip was then trimmed level with a knife so that the lid of the dish was also in contact with the diet. This effectively divided the dish into two and ten or a dozen larvae were then placed on each side of it and the lid was held in place with a rubber band. The larvae were either transferred on hatching, from the nettle leaves used to gather them up from the buddleia and burdock flower heads, or the nettle leaves bearing eggs about to hatch were placed in the dish and the larvae allowed to transfer themselves. This last was much the quickest method. It was found to be not only difficult but very time-consuming and tedious to transfer newly hatched larvae and the discovery that after feeding on nettle they would transfer onto the diet was a great benefit.

The larvae would feed in the petri dishes until they reached the third instar. At this stage the diet became stale, sometimes also mouldy and the larvae in danger of being overcrowded. The second method of presentation was now used. The larva were transferred individually (or sometimes in pairs) into 40 ml clear plastic wide-mouthed vials. The clear, snap-on lids of these were pierced half-a-dozen times with a small awl to give ventilation. A 1½ cm cube of diet was found to last the larva until pupation which took place on the lid of the vial. It was found that while a pair of larvae in each pot could be reared, it needed a double size cube of diet and there also tended to be some disturbance at pupation time.

Initially it was tried putting newly hatched larvae direct into these vials, but, even when several larvae were put into each, it was found that they wandered off the diet and a high mortality resulted. It was fairly obvious that the diet was not an immediate attraction to the larvae, as is nettle, and it is not until the pangs of hunger overcome their desire to search for something better, that they take

to eating it. This applied also to those transferring to the diet after eating plant food. The success in the petri dishes was due to the fact that the diet occupied one entire side of their confined space, floor to ceiling, and consequently their wandering was regularly bringing them into contact with diet, whereas in the vials, once they had left the diet and got up to the lid, it was not easy for them to find their way back down to the cube of diet placed centrally in the base of the vial.

At first the eggbearing florets of burdock and buddleia were placed into 1lb jars, into the bottom of which a 1 cm layer of diet had been poured. Although quite a few of the larvae transferred in this way after hatching onto the diet, they were not happy in this type of container, were also overcrowded and it proved very time-consuming to move them out of the jars onto fresh diet. Also, already mentioned above, serious problems with mould arose. The eventual solution was to place the eggbearing florets into a plastic box and add fresh nettle leaves as the larvae started to hatch. These leaves were then placed into the petri dishes with the diet after the larvae had transferred onto them. Sometimes, for convenience, or because there was not enough diet made up, they were continued to be fed on nettle until 2nd or even 3rd instar. At this stage the larvae are much easier to handle and could be transferred directly into pots containing a cube of diet.

### **The time and cost of using diet**

I have always held that 80% of the cost of maintaining any culture is in keeping the mother stock in being and arises whether or not any insects are being used for any other purpose whatever. It is only if the surplus out-put increases by a factor of five or more that any significant further costs are likely to be incurred. Leaving aside the normal overheads of premises, heating, lighting it is interesting to try and compare the cost of using diet over conventional food-plant. This is no easy sum as a lot will depend on circumstances such as how many other species are also being reared, whether normal foodplant is nearby or has to be fetched from a distance or even bought in.

The actual cost of the diet as described above, taking the prices from the latest catalogues and from a local chemist, works out at £2.20 per litre of diet of which sum half is in the cost of the agar, giving good reason to try experimenting with diet formulae and substituting substances such as gelatine or sodium alginate for the agar, both of which are very much cheaper. It is possible to reduce the quantity of agar and other species have been so reared on diets using the two above mentioned gelling agents or with only half the quantity of agar, but there are difficulties and problems.

Now one litre of diet will support 100 larvae which, if you allow for a few larvae not producing viable pupae, works out at 2½p per butterfly. Compare this with what pupae normally cost to buy. However, also to be added to the cost of production is the sum expended on the plastic containers being used. This can vary from nil, when used yogurt cups etc. can be obtained and pressed into use up to the £35 per thousand for high quality plastic containers. Once obtained however, these can be washed, sterilized and re-used. So the total container cost will depend on how many are needed and how often they are going to be re-used. Against any cost for containers must be offset the saved cost of needing to buy, or perhaps make, conventional type larval cages. In view of the need to keep painted lady larvae at low densities the number of cages required, and hence cost, could be considerable.

Space is also saved by the use of diet. The plastic vials are stacked in plastic seed trays and take up no more room than would a small library of books. Making no smell, or mess, they can also be kept in situations where normal cages would be unacceptable. Indeed a room of 1600 cubic foot can support an output of several thousand per week all the year round. This needs to be compared with perhaps half an acre of greenhouse which is required to produce sufficient natural foodplant throughout the year for a much lower number of butterflies.

The cost of obtaining natural foodplant will depend on circumstances. If on the spot then there is no cost, but if it has to be fetched from some distance then there is the cost of fetching it, both in mileage and time. Unlike natural food also, diet can be guaranteed to be free of accidental contamination by pesticides, a very real hazard for the entomologist today. Most significantly, perhaps, it enables all the year round rearing to be undertaken at a constant cost, whereas to obtain some natural foodplants in winter is both difficult and can involve the very considerable cost of having to heat a glasshouse, or having the food specially flown in from abroad.

The other 'cost' factor to be taken into account is one's time and the answer here is that the greater the number of larvae being reared then the greater is the saving in time over conventional rearing techniques, but for small numbers, dozens certainly, a couple of hundred probably, then diet may well consume more time. The time taken to make a batch of diet is fairly constant, about half an hour, and is independent of the quantity provided a large mixer is available, 201 taking no longer than a 11 batch. Even if only a small mixer is available, then, if done in succession, 4 or 5 one litre batches should take little more than the hour. Once made the diet is storable, so enough to last several weeks can be made up in a single session. Compare this with the time it takes to gather nettle leaves every few days and if a journey also has to be made for them then this

time can be several hours per week. Another aspect insofar as nettle (or thistle) feeding larvae are concerned, is the sheer unpleasantness of gathering such foliage, particularly in wet weather.

The greatest saving in time, however, is in the looking after of the later instars. While it is true that the setting up of individual vials and transferring the larvae into them is more time-consuming than putting nettle leaf into plastic boxes or cages, these larvae on natural food will continue to need feeding every 2 or 3 days, whereas once in their vials those on diet will not. It is in this later stage that the greater the numbers the greater the saving in time. In addition to the time saved another great advantage is that no attention is going to be required over weekends and holidays.

### Discussion

It has been shown that the painted lady butterfly can be readily reared on a semisynthetic diet, albeit that the diet must contain an element of one of the known natural foodplants and the one used, since it is a readily available commercial product, was stinging nettle. It was also shown that low densities in the smaller instars and isolation for the larger larvae gave the maximum survival. This is perhaps not too surprising a result.

The failure to rear on often quoted foodplants, thistles, is, however, both surprising and of considerable import, for it raises some interesting points. It is impossible to believe that all the books are wrong, but two recent papers bear considerable import on this point and also provide a valid explanation not in conflict with the literature.

In the first paper, by Mikheev & Kreslavsky (1980), it has been shown that in the beetle *Lochmaea caprea* there are two distinct races which feed on willow and birch respectively. That on birch is capable of surviving on either foodplant but the willow race can only survive on willow. In nature there is rarely interbreeding and when it does occur the birch addicted race inherits as a dominant allele capable of survival on either foodplant. In *Yponomeuta padellus* too it is known that there are sympatric races preferring separate foodplants. (Emmet, nd., Menken, 1981). Here then we have the mechanism to explain my failure to rear painted ladies on thistle. By chance I had a 'race' fixated on other foodplants and therefore of a genetic makeup which was unable to survive on thistle. I feel this is a valid theory which fits the present facts, although there is as yet no positive proof.

In the second paper, published since the end of this investigation, Warren (1986) observing the painted lady under field conditions discovered that of over 200 larvae found on thistle only

one was on spear thistle (*C. vulgare*) and none at all on creeping thistle (*C. arvense*) although both these species were in abundance. The larvae in fact were on musk thistle (*C. nutans*) a species I did not try and which is not so often quoted in the literature as are the others. It is, I feel, highly significant that although so plentiful, less than half a percent of the other thistle species (a single example only in fact) had a larva on it. What we do not know of course is how many ova were deposited on these other thistles from which the larvae failed to survive and there is clearly scope for considerably more observations to be made on the oviposition choices and survival rate of painted lady larvae on various foodplants.

For the laying of ova on clearly lethal plants as buddleia and *Lavatera* I have no explanation. It could be a facet of cage confinement or there may even be 'races' of the painted lady that can survive on these plants. The laying on *Lavatera* is more understandable than on buddleia, for it is related to a suitable foodplant, *Malva*, and almost certainly contains the same chemical laying cue.

For some as yet unexplained reason, the larvae of butterflies (Papilionoidea) require their diet to contain an element of the natural foodplant (or its flavouring essence), whereas most other Lepidoptera do not and their larvae will happily take to a neutral flavour and often more simply compounded diet. In this aspect the painted lady is no exception, required the addition of dried nettle to the diet and failed on the neutral diet tried.

The successful proof that the painted lady can be reared on diet means that it is now possible for them to be reared on a very large scale all the year round without the use of large and expensive glasshouses and their comparatively short life-cycle makes them an ideal insect for teaching and research. It is possible that a limiting factor may be in keeping the adults fertile through the winter months, but while I lost these during the snows of February, I remain convinced that given the right facilities this problem can be overcome. It also seems highly probable that other nettle feeding species will be equally easy to rear on the same diet.

The most surprising aspect discovered was the facility with which larvae already feeding on nettle would switch to diet. This is known not to occur with quite a few other species, such as *P. brassicae* and *Manduca sexta* and was a bonus which simplifies the rearing procedure. I cannot offer any explanation for this and it is interesting to speculate if it might occur in other Nymphalidae.

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THE TRUE DATE OF PUBLICATION OF F. W. FROHAWK'S NATURAL HISTORY OF BRITISH BUTTERFLIES. — I was sorry to see the date of this work again incorrectly given as 1914 in Colin Pratt's recent history of the comma butterfly in this journal. I have seen this incorrect date quoted before; it seems to be a commonly held view and I believe it has arisen due to persistent misquotes in bookdealers catalogues. The true date of publication is 1924 and I am of the firm opinion that this needs to be put on record in print in order to avoid future errors. As an example, a ten-year misquote in the revised Kloet and Hincks (1854 for 1845) led to a mistaken change in nomenclature, now reversed (Emmet *Entomologist's Gaz.* **38**: 66).

A study of the contents of the two volumes of *The Natural History of British Butterflies* reveal the life-history of the large blue butterfly, which, and I believe this is a well-known fact, was not discovered until 1915. Data for specimens on some of the plates are given as 1919 and the 1920's. This alone makes a nonsense of a publication date of 1914. In the *Entomologist* for February 1923 we have a note about the work from which I quote:— "But if the two volumes, when published . . . ." "The size of the work will be. . ." Two years later we are given a full review (*Entomologist* **63**: 43-45) where the date of publication is unequivocally stated as 1924, and with 60 coloured and 5 plain plates. Curiously, the half page advert for the book in the same issue only mentions 4 plain plates. In dealers catalogues I have seen this number to vary and even seen 1925 (as well as 1914) given as the publication date. — BRIAN O. C. GARDINER, 18 Chesterton Hall Crescent, Cambridge, CB2 1AP.

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ALOEIDES PALLIDA JONATHANI PRINGLE: DESIGNATION OF ALLOTYPE — in the original description of this subspecies (*Ent. Rec.* **99**: 4-6), the designation of the allotype was omitted, and should be as follows:

ALLOTYPE : Kammanassie Mountain 24/12/1978 (Dr. J. B. Ball).